Technical note

Ultraminiature stimulus-isolation probe*

Keywords—*Optical isolation, Stimulation*

Introduction

WITH many electrophysiological experiments it is necessary to initiate the electrophysiological process to be studied artificially. For this purpose, a current has to be injected into nerve or muscle tissue by means of two electrodes (KAHN and MAVEUS, 1972; MANSFIELD, 1967).

The main problems occurring with artificial stimulation are

(*a*) the stimulation has to be local;

(*b*) undesirable electrochemical electrode reactions at the stimulus electrode-tissue interface have to be prevented;

(*c*) a stimulus artefact in the measured electrical response has to be prevented.

The first two problems depend on the size and shape of the stimulus electrodes and the accessibility of the fibres to be stimulated, which determines the necessary current density of the stimulus current. The prevention of undesirable electrochemical electrode reactions can easily be controlled by measuring the stimulus-electrode potential during current flow (BERGVELD, 1976). The third problem, the prevention of a stimulus artefact, will be described in this paper. It appears that by using an optically coupled ultraminiature stimulation circuit built as a probe containing the actual stimulus electrodes, and also using a suitable configuration of the measuring electrodes, the stimulus artefact can be cancelled out completely.

General principle of artificial stimulation

In general, the performance of an electrophysiological measurement consists of the use of a metal or glass-pipette microelectrode connected to the high-impedance input lead of an amplifier circuit, while the second input lead of the amplifier is connected to a reference electrode which is usually the earthed lead of the complete measuring equipment. It will be obvious that stimulating by means of two electrodes, connected to an earthed stimulator equipment will now cause large artefacts.

This problem can be solved by disconnecting the stimulation unit from the earthed system by means of a conversion to a floating output circuit. This idea has already resulted in commercially available so-called stimulus isolators. However, in practice, it appears that it is still difficult to prevent stimulus artefacts, because, as will be shown in Section 4 of this paper, the capacitance of the floating part of the stimulation circuit to earth has to be smaller than 1 pF, which is usually not the case with the commercially available stimulus-isolator units. In the following Section we will therefore describe a battery-powered, optical-coupled stimulator circuit which has such a small volume that the capacitance to earth is definitely smaller than 1 pF. Using this circuit the

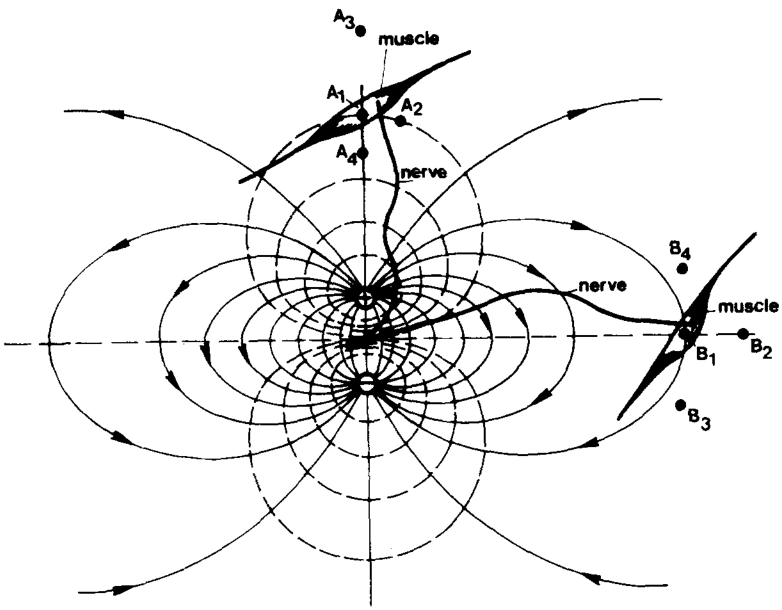

Fig. 1 Theoretical model of current lines and equipotential lines around two stimulus electrodes, with the points of measurement as mentioned in the text
——— current lines
– – – equipotential lines

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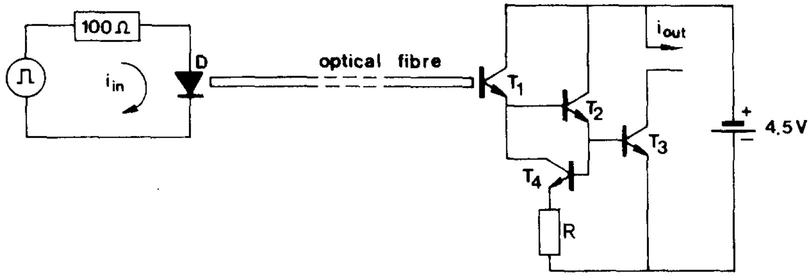

Fig. 2a Circuit diagram of complete stimulator
D = l.e.d.
T_1 = LS600 Texas Instruments
$T_2 = T_3 = T_4$ = BCW33 Philips
R = 33 kΩ pin-head resistor 0·05W

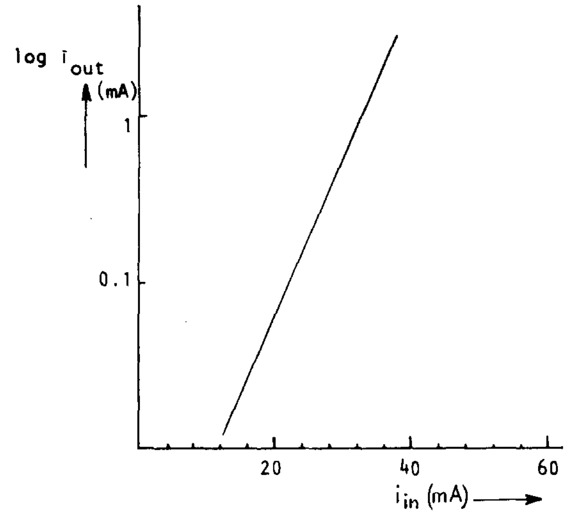

Fig. 2b Stimulator output current i_{out} as a function of the current i_{in} through the l.e.d.

capacitive leakage current to earth is so small that no artefact will be measurable.

However, there is still another reason why an artefact can occur. During stimulation a current will flow from one of the stimulus electrodes to the other through the tissue and the surrounding fluid. This also results in a current flow through the area where the measurement is performed. Therefore, besides the evoked response, a direct contribution of this current flow will also be measured, resulting in an artefact. This artefact can only be prevented by a proper configuration of the two electrodes of the measuring circuit. While the microelectrode is placed at the point where a response will be measured, the reference electrode will, in general, be placed at some distance elsewhere in the surrounding fluid at an electrophysiologically inactive place. There are, however, places where the stimulus current flow results in the same potential as that at the position of the microelectrode. This can easily be seen in a theoretical model as given in Fig. 1, where a pair of stimulus electrodes are sketched, with the corresponding current lines and equipotential lines. It is assumed that stimulation occurs in the region between the stimulus electrodes (strongest field), and that A_1 and B_1 are, for example, the points where the electrical response is measured. The electrophysiologically inactive points A_2 and B_2 are of the same potential as A_1 and B_1, respectively. The reference electrodes have therefore to be placed at these points to prevent a stimulus artefact. Placing the reference electrodes at points A_3 and B_3 will cause a positive artefact and at the points A_4 and B_4 a negative artefact.

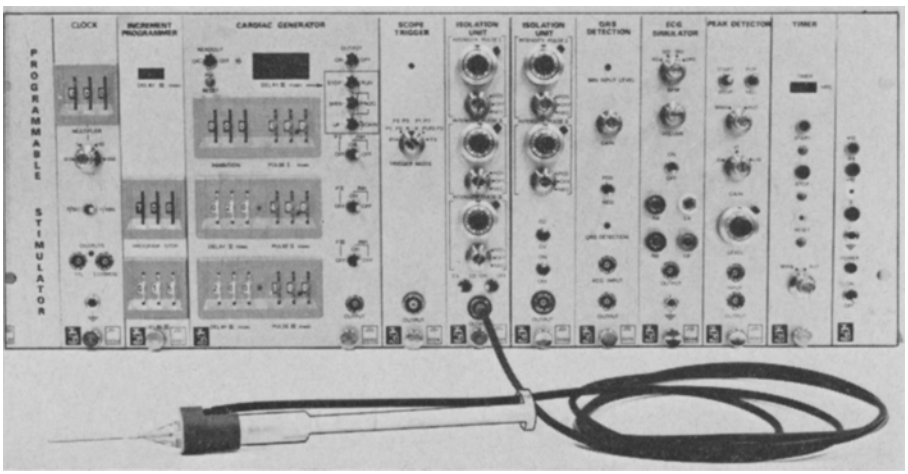

Fig. 3 Stimulus isolation probe, attached to a programmable stimulator of Janssen Scientific Instruments with l.e.d. output, by means of an optical fibre, with a length of 2m

In practice, it appears that the electric field is not as homogeneous as is assumed in the theoretical model, but the points of equipotential can easily be found by manipulating the reference electrode around the micro-electrode, if the measuring electrodes are placed approximately in a plane perpendicular to the direction of the inserted stimulus electrodes.

Summarising, it may be concluded that two contributions to a stimulus artefact can be distinguished, one caused by a leakage current to the earthed lead of the measuring circuit and the other caused by current flow through the area in which the measurement has been carried out. The first contribution can be cancelled out by using an actual floating stimulator circuit, the second by proper positioning of the reference electrode of the measuring equipment.

Stimulator circuit and probe performance

The starting points for the development of the stimulator are, as is obvious from the previous Section, that the total volume of the stimulator must be as small as possible to reduce the capacitance to earth to a minimum. This means that the actual stimulator should have a minimum of components; thus it should not be a complete generator but only a battery-powered convertor; as such it would be able to convert an optical signal into a stimulus current. The small convertor can then be built as a probe to which the stimulation electrodes can be attached directly, for instance, by inserting them into a pair of hypodermic needles. This circuit has to be optically coupled with a light-emitting diode (l.e.d.), which can easily be done by means of a thin flexible optical fibre

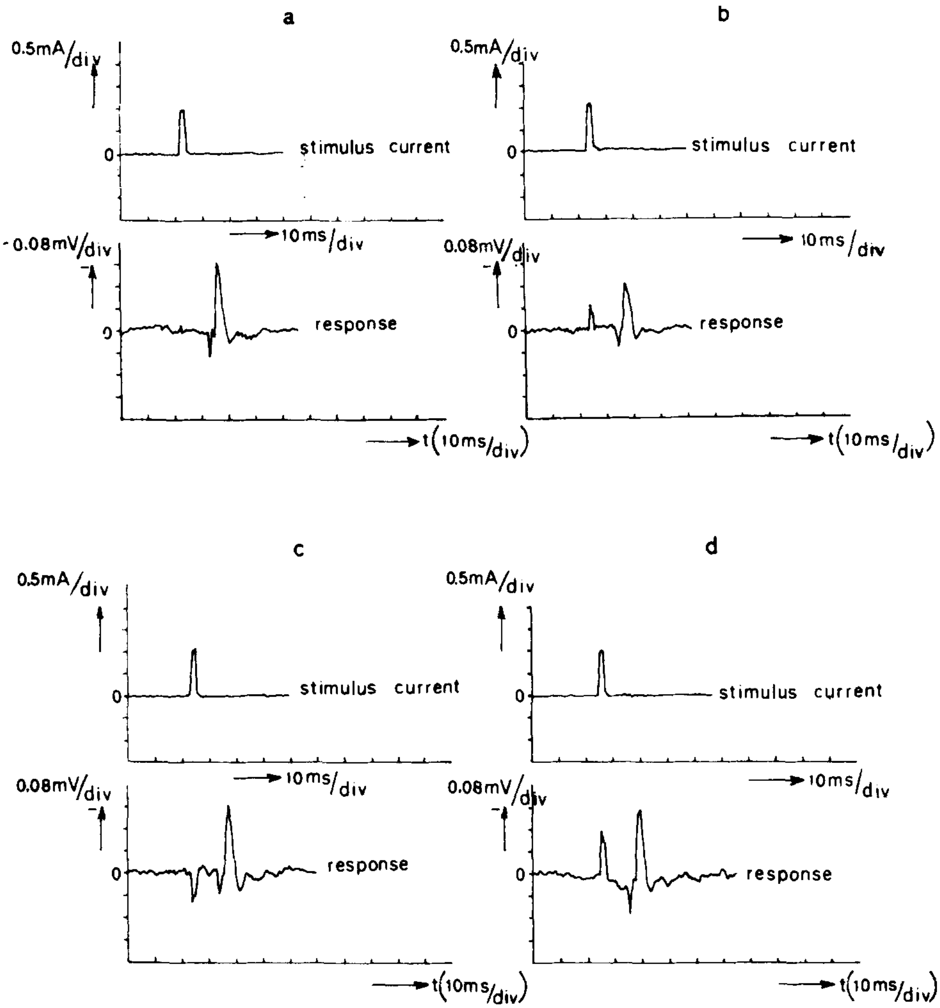

Fig. 4 (a) Microelectrode and reference electrode at equipotential
No stimulus artefact occurs
(b) As for Fig. 4(a), but stimulator is connected to earth by a capacitor of 1·5 pF
A stimulus artefact occurs
(c) Microelectrode and reference electrode not at equipotential
A positive artefact occurs
(d) As for Fig. 4(c), but with a negative artefact

The l.e.d. has to be operated by a normal pulse generator used for artificial stimulation, placed outside the Faraday-cage in which the measurement is carried out.

Besides the requirement of a minimum volume, it is preferable that the circuit does not use a bias current, so that no current is drawn from the batteries when no stimulation pulse is generated, also for the reason that no direct current may flow through the electrodes. Finally, the stimulator has to be a current source, which can generate pulses of 100 μs to 1 ms in the range of 0·03 mA to 3 mA using electrodes with tip areas of about 0·5 mm^2. The circuit is shown in Fig. 2A in which T_1 is a phototransistor, T_2 and T_3 a Darlington pair, while T_4 gives a feedback to speed up the rise time of the system. D is the light-emitting diode which can be supplied by a normal earthed stimulator. The conversion of the current through the l.e.d. into the current through the electrodes is given in Fig. 2B.

The probe performance is shown in Fig. 3. The stimulation electrodes consist of glass-insulated platinum electrodes, glued to each other, which can be easily attached to the hypodermic needles of the probe head. This probe head contains the electronic circuit, which consists of a printed circuit 7·5 mm in diameter, to which the optical fibre can be attached through a hole in the small protrusion. The batteries are encased in the shaft of the manipulator attachment, which can be screwed onto the probe head. These batteries are three Mallory cells, type MS-13H, diameter 7·75 mm, length 5·21 mm. This means that the volume of the stimulator, and thus the capacitance to earth is determined by the area of the batteries.

Results

In practice, the probe is tested, by stimulation of the N5 nerve of a locust, while the electrical response at the flexor tibialis muscle fibres is measured, using an f.e.t. measuring probe with a 6 μm platinum microelectrode [Bergveld (1968)].

The pulse width was 1 ms during this experiment, but it can be decreased to 200 μs, with a rise time of 100 μs and a fall time of 20 μs. It appears that no measurable leakage current to earth can be detected if the microelectrode and the reference electrode are at equipotential (Fig. 4*a*).

With a 1·5 pF capacitor connected between the positive stimulation electrode and earth, a stimulus artefact already occurs (Fig. 4*b*), which means that the capacitance between the probe itself and earth, in fact the capacitance between the battery cases and earth, is smaller than 1 pF. As will be obvious, the stimulus artefact increases if higher shunt capacitors are used. This demonstrates the effect of the 'floating' requirements. Also, the effect of the configuration of the measuring electrode can be readily demonstrated. If in the situation of Fig. 4*a* the reference electrode is moved to another position, positive or negative artefacts can be observed (Fig. 4*c* and *d*), in agreement with the theoretical expectation on the grounds of the current flow diagram of Fig. 1.

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The stimulus isolation probe described in this paper will be manufactured by Janssen Scientific Instruments 2340 Beerse, Belgium